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Økonomi- og Erhvervsministeriet

28 April 2005

Pia Høybye-Olsen

PATENT- OG VAREMÆRKESTYRELSEN

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DIPHENYL OX-INDOL-2-ON COMPOUNDS AND THEIR USE IN THE TREATMENT OF CANCER

FIELD OF THE INVENTION

The present invention relates to substituted 3,3-Diphenyl-1,3-dihydro-indol-2-one compounds.

BACKGROUND OF THE INVENTION

US 1,624,675 describes O-O-diacyl derivatives of diphenolisatine and that these compounds posses laxative properties.

While inhibition of protein synthesis inhibits cell proliferation, highly proliferative cancer cells may be more sensitive than normal cells to protein synthesis inhibition because many oncogenes and growth regulatory proteins required for effective cell proliferation are encoded by inefficiently translated mRNAs, and are dependent on eukaryotic translation initiation factors (Aktas et al (1998) Proc Natl Acad Sci 95, 8280 and references therein).

Protein synthesis is regulated in response to cell stress, which can be induced by environmental or physiological challenges (such as hypoxia, amino acid or nutrient deprivation), intracellular calcium load and protein glycosylation inhibition. For example, cell stressors such as clotrimazole, 3,3-diphenyloxindole, thapsigargin, tunicamycin and arsenite (Aktas et al (1998) Proc Natl Acad Sci 95, 8280; Brewer et al (1999) Proc Natl Acad Sci 96, 8505-8510; Harding et al (2000) Molecular Cell 5, 897-904; Natarajan et al (2004) J Med Chem 47, 1882-1885) act as translation initiation inhibitors, reducing both protein synthesis and cell proliferation.

The possibility that translation initiation inhibiters may have potential as anti-cancer drugs has been described previously (Aktas et al (1998) Proc Natl Acad Sci 95, Natarajan et al (2004) J.Med.Chem 47, 1882-1885). The Natarajan paper further disclose 3,3-Diaryl-1,3-dihydroindol-2-ones which potentially inhibit translation initiation.

Protein synthesis is also regulated by the mTOR pathway, providing another link a nutrient and amino acid status (Harris & Lawrence (2003) ScienceSTKE (212) re15; Nave et al (1999) Biochem J 344, 427; Beaunet et al (2003) Biochem J 372, 555-566; Inoki et al (2003) Cell 115, 577-590). This pathway is also linked to regulation of the translation initiation complex (Cherkasova & Hinnebusch (2003) Genes & Dev 17, 859-872; Kubota et al (2003) J Biol Chem 278, 20457). Inhibition of mTOR signalling inhibits the proliferation of cancer cell lines (Noh et al (2004) Clinical Cancer Research 10, 1013-1023; Yu et al (2001) Endocrine-Related Cancer 8, 249-258), and has been proposed as a target for cancer therapy (Huang & Houghton (2003) Curr Opin Pharmacol 3, 371-377).

However, there is still a need for compounds capable of inhibiting the uncontrolled growth of cancer cells.

SUMMARY OF THE INVENTION

Compounds of general formula (I) are shown to inhibit the proliferation of MDA468 cells at lower concentrations as those required to inhibit proliferation of MDA231 cells. A possible mechanism to explain this finding is the selective inhibition of protein synthesis by compounds of general formula (I) in MDA468 cells compared to MDA231 cells. Our present hypothesis is that compounds of the general formula (I) inhibit protein synthesis by selective inhibition of mTOR pathway activation of translation inhibition.

The examples illustrate how the compounds can be synthesized.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Results from the cell proliferation studies

Figure 2: Results from the cell proliferation studies

Figure 3: Results from the cell proliferation studies

Figure 4: Results of the protein synthesis experiments.

Figure 5: Translational Control

Figure of Cell Signaling technology catalog 2003-2004.

Figure 6: Western Blots - MDA468 Cells (24 hour compound incubation)

Figure 7: Western Blots - Comparison of MDA468 & MDA 231 cells (48 hours incubation)

Figure 8: Xenograft

DETAILED DESCRIPTION OF THE INVENTION

The term cancer is typically describing cell growth not under strict control. In one embodiment of the invention treatment of cancers in which inhibition of protein synthesis and/or inhibition of activation of the mTOR pathway is an effective method for reducing cell growth. Examples of such cancers are breast cancer, renal cancer, multiple myeloma, leucemia, glia blastoma, rhabdomyosarcoma, prostate, soft tissue sarcoma, colorectal sarcoma, gastric carcinoma, head and neck squamous cell carcinoma, uterine, cervical, melanoma, lymphoma, and pancreatic.

The selective inhibition of mTOR pathway activation by compounds of the general formula (I) in Western blots correlates with cell proliferation and protein synthesis data. This suggests that detection of mTOR pathway activity by measurement of either p70S6K, 4E-BP1 or S6K phosphorylation status using phosphor-specific or total protein antibodies by Western blot or ELISA, or measurement of p70S6K kinase activity, in patient tumour material or blood samples, may provide a useful method for selecting patients who will respond to compounds of general formula (I). Alternatively, measurement of p70S6K or S6K phosphorylation status using phosphospecific antibodies, or p70S6K kinase activity, in tumour material or blood samples may provide a biomarker useful for determining drug dosing of compounds of the general formula (I) in human clinical trials.

In the present context, the term "C_{1-6}-alkyl" is intended to mean a linear, cyclic or branched hydrocarbon group having 1 to 6 carbon atoms, such as methyl, ethyl, propyl, *iso*-propyl, pentyl, cyclopentyl, hexyl, cyclohexyl, and the term "C_{1-4}-alkyl" is intended to cover linear, cyclic or branched hydrocarbon groups having 1 to 4 carbon atoms, e.g. methyl, ethyl, propyl, *iso*-propyl, cyclopropyl, butyl, *iso*-butyl, *tert*-butyl, cyclobutyl.

Similarly, the term "C_{2-6}-alkenyl" is intended to cover linear, cyclic or branched hydrocarbon groups having 2 to 6 carbon atoms and comprising one unsaturated bond. Examples of alkenyl groups are vinyl, allyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, heptadecaenyl. Preferred examples of alkenyl are vinyl, allyl, butenyl, especially allyl.

In the present context, i.e. in connection with the terms "alkyl", "alkoxy", and "alkenyl", , the term "optionally substituted" is intended to mean that the group in question may be substituted one or several times, preferably 1-3 times, with group(s) selected from hydroxy (which when bound to an unsaturated carbon atom may be present in the tautomeric keto form), C_{1-6}-alkoxy (i.e. C_{1-6}-alkyl-oxy), C_{2-6}-alkenyloxy, carboxy, oxo (forming a keto or aldehyde functionality), C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxy, arylamino, arylcarbonyl, aryloxycarbonyl, arylcarbonyloxy, arylaminocarbonyl, arylcarbonylamino, heteroaryl, heteroaryloxy, heteroarylamino, heteroarylcarbonyl, heteroaryloxycarbonyl, heteroarylcarbonyloxy, heteroarylaminocarbonyl, heteroarylcarbonylamino, heterocyclyl, heterocyclyloxy, heterocyclylamino, heterocyclylcarbonyl, heterocyclyloxycarbonyl, heterocyclylcarbonyloxy, heterocyclylaminocarbonyl, heterocyclylcarbonylamino, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, C_{1-6}-alkylcarbonylamino, cyano, guanidino, carbamido, C_{1-6}-alkyl-sulphonyl-amino, aryl-sulphonyl-amino, heteroaryl-sulphonyl-amino, C_{1-6}-alkanoyloxy, C_{1-6}-alkyl-sulphonyl, C_{1-6}-alkyl-sulphinyl, C_{1-6}-alkylsulphonyloxy, nitro, C_{1-6}-alkylthio, and halogen, where any aryl, heteroaryl and heterocyclyl may be substituted as specifically described below for aryl, heteroaryl and heterocyclyl, and any alkyl, alkoxy, and the like, representing substituents may be substituted with hydroxy, C_{1-6}-alkoxy, amino, mono- and di(C_{1-6}-alkyl)amino, carboxy, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylaminocarbonyl, or halogen(s).

Typically, the substituents are selected from hydroxy (which when bound to an unsaturated carbon atom may be present in the tautomeric keto form), C_{1-6}-alkoxy (i.e. C_{1-6}-alkyl-oxy), C_{2-6}-alkenyloxy, carboxy, oxo (forming a keto or aldehyde functionality), C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxy, arylamino, arylcarbonyl, heteroaryl, heteroaryloxy, heteroarylamino, heteroarylcarbonyl, heterocyclyl, heterocyclyloxy, heterocyclylamino, heterocyclylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino; carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkylcarbonylamino, guanidino, carbamido, C_{1-6}-alkyl-sulphonyl-amino, C_{1-6}-alkyl-sulphonyl, C_{1-6}-alkyl-sulphinyl, C_{1-6}-alkylthio, halogen, where any aryl, heteroaryl and heterocyclyl may be substituted as specifically described below for aryl, heteroaryl and heterocyclyl.

In some embodiments, substitutents are selected from hydroxy, C_{1-6}-alkoxy, amino, mono- and di(C_{1-6}-alkyl)amino, carboxy, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylaminocarbonyl, or halogen.

The term "Halogen" includes fluoro, chloro, bromo, and iodo.

In the present context, the term "aryl" is intended to mean a fully or partially aromatic carbocyclic ring or ring system, such as phenyl, naphthyl, 1,2,3,4-tetrahydronaphthyl, anthracyl, phenanthracyl, pyrenyl, benzopyrenyl, fluorenyl and xanthenyl, among which phenyl is a preferred example.

The term "heteroaryl" is intended to mean a fully or partially aromatic carbocyclic ring or ring system where one or more of the carbon atoms have been replaced with heteroatoms, e.g. nitrogen (=N- or –NH-), sulphur, and/or oxygen atoms. Examples of such heteroaryl groups are oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, coumaryl, furanyl, thienyl, quinolyl, benzothiazolyl, benzotriazolyl, benzodiazolyl, benzooxozolyl, phthalazinyl, phthalanyl, triazolyl, tetrazolyl, isoquinolyl, acridinyl, carbazolyl, dibenzazepinyl, indolyl, benzopyrazolyl, phenoxazonyl. Particularly interesting heteroaryl groups are benzimidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, furyl, thienyl, quinolyl, triazolyl, tetrazolyl, isoquinolyl, indolyl in particular benzimidazolyl, pyrrolyl, imidazolyl, pyridinyl, pyrimidinyl, furyl, thienyl, quinolyl, tetrazolyl, and isoquinolyl.

The term "heterocyclyl" is intended to mean a non-aromatic carbocyclic ring or ring system where one or more of the carbon atoms have been replaced with heteroatoms, e.g. nitrogen (=N- or –NH-), sulphur, and/or oxygen atoms. Examples of such heterocyclyl groups (named according to the rings) are imidazolidine, piperazine, hexahydropyridazine, hexahydropyrimidine, diazepane, diazocane, pyrrolidine, piperidine, azepane, azocane, aziridine, azirine, azetidine, pyroline, tropane, oxazinane (morpholine), azepine, dihydroazepine, tetrahydroazepine, and hexahydroazepine, oxazolane, oxazepane, oxazocane, thiazolane, thiazinane, thiazepane, thiazocane, oxazetane, diazetane, thiazetane, tetrahydrofuran, tetrahydropyran, oxepane, tetrahydrothiophene, tetrahydrothiopyrane, thiepane, dithiane, dithiepane, dioxane, dioxepane, oxathiane, oxathiepane. The most interesting examples are tetrahydrofuran, imidazolidine, piperazine, hexahydropyridazine, hexahydropyrimidine, diazepane, diazocane, pyrrolidine, piperidine, azepane, azocane, azetidine, tropane, oxazinane (morpholine), oxazolane, oxazepane, thiazolane, thiazinane, and thiazepane, in particular tetrahydrofuran, imidazolidine, piperazine, hexahydropyridazine, hexahydropyrimidine, diazepane, pyrrolidine, piperidine, azepane, oxazinane (morpholine), and thiazinane.

In the present context, i.e. in connection with the terms "aryl", "heteroaryl", "heterocyclyl" and the like (e.g. "aryloxy", "heterarylcarbonyl", etc.), the term "optionally substituted" is intended to mean that the group in question may be substituted one or several times, preferably 1-5 times, in particular 1-3 times, with group(s) selected from hydroxy (which when present in an enol system may be represented in the tautomeric keto form), C_{1-6}-alkyl, C_{1-6}-alkoxy, C_{2-6}-alkenyloxy, oxo (which may be represented in the tautomeric enol form), carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxy, arylamino, aryloxycarbonyl, arylcarbonyl, heteroaryl, heteroarylamino, amino, mono- and di(C_{1-6}-alkyl)amino; carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkylcarbonylamino, cyano, guanidino, carbamido, C_{1-6}-alkanoyloxy, C_{1-6}-alkyl-sulphonyl-amino, aryl-sulphonyl-amino, heteroaryl-sulphonyl-amino, C_{1-6}-alkyl-suphonyl, C_{1-6}-alkyl-sulphinyl, C_{1-6}-alkylsulphonyloxy, nitro, sulphanyl, amino, amino-sulfonyl, mono- and di(C_{1-6}-alkyl)amino-sulfonyl, dihalogen-C_{1-4}-alkyl, trihalogen-C_{1-4}-alkyl, halogen, where aryl and heteroaryl representing substituents may be substituted 1-3 times with C_{1-4}-alkyl, C_{1-4}-alkoxy, nitro, cyano, amino or halogen, and any alkyl, alkoxy, and the like, representing substituents may be substituted with hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkenyloxy, amino, mono- and di(C_{1-6}-alkyl)amino, carboxy, C_{1-6}-alkylcarbonylamino, halogen, C_{1-6}-alkylthio, C_{1-6}-alkyl-sulphonyl-amino, or guanidino.

Typically, the substituents are selected from hydroxy, C_{1-6}-alkyl, C_{1-6}-alkoxy, oxo (which may be represented in the tautomeric enol form), carboxy, C_{1-6}-alkylcarbonyl, formyl, amino, mono- and di(C_{1-6}-alkyl)amino; carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkylcarbonylamino, guanidino, carbamido, C_{1-6}-alkyl-sulphonyl-amino, aryl-sulphonyl-amino, heteroaryl-sulphonyl-amino, C_{1-6}-alkyl-suphonyl, C_{1-6}-alkyl-sulphinyl, C_{1-6}-alkylsulphonyloxy, sulphanyl, amino, amino-sulfonyl, mono- and di(C_{1-6}-alkyl)amino-sulfonyl or halogen, where any alkyl, alkoxy and the like, representing substituents may be substituted with hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkenyloxy, amino, mono- and di(C_{1-6}-alkyl)amino, carboxy, C_{1-6}-alkylcarbonylamino, halogen, C_{1-6}-alkylthio, C_{1-6}-alkyl-sulphonyl-amino, or guanidino. In some embodiments, the substituents are selected from C_{1-6}-alkyl, C_{1-6}-alkoxy, amino, mono- and di(C_{1-6}-alkyl)amino, sulphanyl, carboxy or halogen, where any alkyl, alkoxy and the like, representing substituents may be substituted with hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkenyloxy, amino, mono- and di(C_{1-6}-alkyl)amino, carboxy, C_{1-6}-alkylcarbonylamino, halogen, C_{1-6}-alkylthio, C_{1-6}-alkyl-sulphonyl-amino, or guanidino.

The compounds for use as a medicament are typically formulated in a pharmaceutical composition prior to use.

The administration route of the compounds may be any suitable route which leads to a concentration in the blood or tissue corresponding to a therapeutic effective concentration. Thus, e.g., the following administration routes may be applicable although the invention is not limited thereto: the oral route, the parenteral route, the cutaneous route, the nasal route, the rectal route, the vaginal route and the ocular route. It should be clear to a person skilled in the art that the administration route is dependent on the particular compound in question, particularly the choice of administration route depends on the physico-chemical properties of the compound together with the age and weight of the patient and on the particular disease or condition and the severity of the same.

The compounds may be contained in any appropriate amount in a pharmaceutical composition, and are generally contained in an amount of about 1-95%, e.g. 1-10%, by weight of the total weight of the composition. The composition may be presented in a dosage form which is suitable for the oral, parenteral, rectal, cutaneous, nasal, vaginal and/or ocular administration route. Thus, the composition may be in form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, delivery devices, suppositories, enemas, injectables, implants, sprays, aerosols and in other suitable form.

The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice, see, e.g., "Remington's Pharmaceutical Sciences" and "Encyclopedia of Pharmaceutical Technology", edited by Swarbrick, J. & J. C. Boylan, Marcel Dekker, Inc., New York, 1988. Typically, the compounds defined herein are formulated with (at least) a pharmaceutically acceptable carrier or excipient. Pharmaceutically acceptable carriers or excipients are those known by the person skilled in the art. Formation of suitable salts of the compounds of the Formula I will also be evident in view of the before-mentioned.

Thus, the present invention provides in a further aspect a pharmaceutical composition comprising a compound of the general Formula I in combination with a pharmaceutically acceptable carrier.

Pharmaceutical compositions according to the present invention may be formulated to release the active compound substantially immediately upon administration or at any substantially predetermined time or time period after administration. The latter type of compositions is generally known as controlled release formulations.

In the present context, the term "controlled release formulation" embraces i) formulations which create a substantially constant concentration of the drug within the body over an extended period of time, ii) formulations which after a predetermined lag time create a substantially constant concentration of the drug within the body over an extended period of time, iii) formulations which sustain drug action during a predetermined time period by maintaining a relatively, constant, effective drug level in the body with concomitant minimization of undesirable side effects associated with fluctuations in the plasma level of the active drug substance (sawtooth kinetic pattern), iv) formulations which attempt to localize drug action by, e.g., spatial placement of a controlled release composition adjacent to or in the diseased tissue or organ, v) formulations which attempt to target drug action by using carriers or chemical derivatives to deliver the drug to a particular target cell type.

Controlled release formulations may also be denoted "sustained release", "prolonged release", "programmed release", "time release", "rate-controlled" and/or "targeted release" formulations.

Controlled release pharmaceutical compositions may be presented in any suitable dosage forms, especially in dosage forms intended for oral, parenteral, cutaneous nasal, rectal, vaginal and/or ocular administration. Examples include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, microspheres, nanoparticles, liposomes, delivery devices such as those intended for oral, parenteral, cutaneous, nasal, vaginal or ocular use.

Preparation of solid dosage forms for oral use, controlled release oral dosage forms, fluid liquid compositions, parenteral compositions, controlled release parenteral compositions, rectal compositions, nasal compositions, percutaneous and topical compositions, controlled release percutaneous and topical compositions, and compositions for administration to the eye will be well-known to those skilled in the art of pharmaceutical formulation. Specific formulations can be found in "Remington's Pharmaceutical Sciences".

Capsules, tablets and pills etc. may contain for example the following compounds: microcrystalline cellulose, gum or gelatin as binders; starch or lactose as excipients;

stearates as lubricants; various sweetening or flavouring agents. For capsules the dosage unit may contain a liquid carrier like fatty oils. Likewise coatings of sugar or enteric agents may be part of the dosage unit. The pharmaceutical compositions may also be emulsions of the compound(s) and a lipid forming a micellular emulsion.

For parenteral, subcutaneous, intradermal or topical administration the pharmaceutical composition may include a sterile diluent, buffers, regulators of tonicity and antibacterials. The active compound may be prepared with carriers that protect against degradation or immediate elimination from the body, including implants or microcapsules with controlled release properties. For intravenous administration the preferred carriers are physiological saline or phosphate buffered saline.

The compound are preferably administered in an amount of about 0.1-200 mg per kg body weight per day, such as about 0.5-25 mg per kg body weight per day.

For compositions adapted for oral administration for systemic use, the dosage is normally 2 mg to 1 g per dose administered 1-4 times daily for 1 week to 12 months depending on the disease to be treated.

The dosage for oral administration of the composition in order to prevent diseases or conditions is normally 0.1 mg to 200 mg per kg body weight per day. The dosage may be administered once or twice daily for a period starting 1 week before the exposure to the disease until 4 weeks after the exposure.

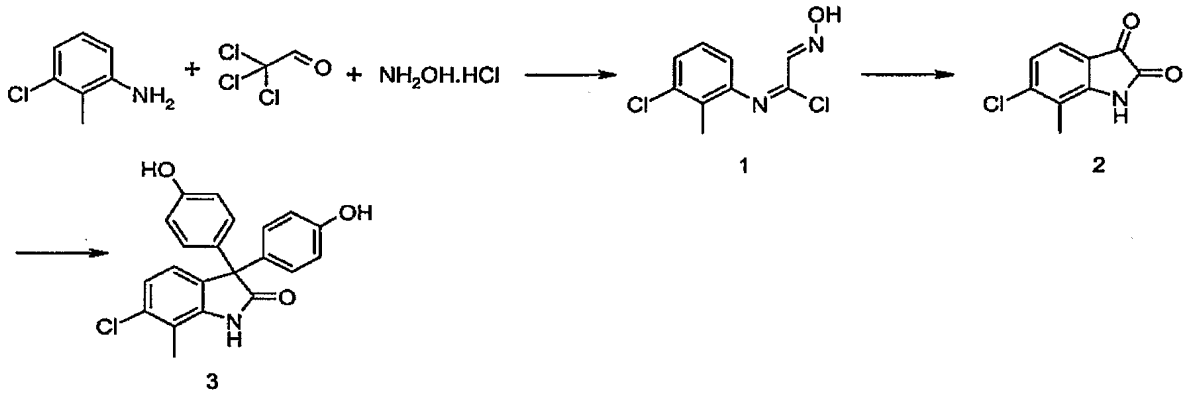
For compositions adapted for rectal use for preventing diseases, a somewhat higher amount of the compound is usually preferred, i.e. from approximately 1 mg to 100 mg per kg body weight per day.

For parenteral administration, a dose of about 0.1 mg to about 200 mg per kg body weight per day is convenient. For intravenous administration a dose of about 0.1 mg to about 200 mg per kg body weight per day administered for 1 day to 3 months is convenient. For intraarticular administration a dose of about 0.1 mg to about 200 mg per kg body weight per day is usually preferable. For parenteral administration in general, a solution in an aqueous medium of 0.5-2% or more of the active ingredients may be employed.

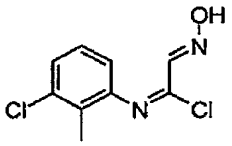
For topical administration on the skin, a dose of about 1 mg to about 5 g administered 1-10 times daily for 1 week to 12 months is usually preferable.

EXAMPLES

Example 1: Synthetic route followed for 6-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one (3)



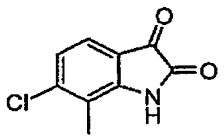
N-(3-Chloro-2-methyl-phenyl)-2-hydroxyimino-acetimidoyl chloride (1)



To a well stirred suspension of sodium sulfate (314.g, 2211 mmol) in water (700 mL) at 60°C were added in sequence hydroxylamine hydrochloride (56 g, 806 mmol), chloral hydrate (47 g, 284 mmol), 2-methyl-3-chloro-aniline (40 g, 283 mmol) in water (500 mL) and finally concentrated hydrochloric acid (12 M, 24.2 ml, 290 mmol). The mixture temperature was risen to 100°C. After 20 minutes the brown solution was left to cool to RT and kept stirring overnight. The solid present was filtered, washed with water (3X), heptane (2X) and dried at 60°C under vacuum for 6 h. Obtained 62 g of N-(3-Chloro-2-methyl-phenyl)-2-hydroxyimino-acetimidoyl chloride (1) as a beige solid used without further purification.

δ_H (400 MHz, DMSO-d6) 12.3 (1 H, s), 9.8 (1 H, s), 7.7 (1 H, s), 7.42 (1 H, d, *J*= 7.8), 7.36 (1 H, d, *J*= 7.6), 7.3 (1 H, m), 2.25 (3 H, s).

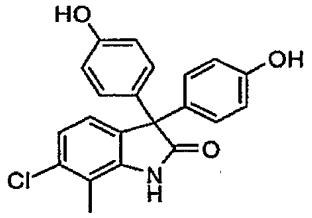
6-Chloro-7-methyl-1H-indole-2,3-dione (2)



To well stirred sulphuric acid (18.3 M, 300 ml) heated at 50°C was added 1 in small portion over 20 minutes (exothermic up to 70°C) (60 g, 282 mmol). After addition was completed the temperature was risen to 80°C and kept for 20 minutes after which the reaction was left cool to RT. The brown mixture was slowly poured into ice (~500 g) and water (500 mL), diluted with more water (1 L) to yield a brown-orange slurry. The solid was collected by filtration, washed with water (2X) under suction to yield an orange solid. This solid was dissolved in 0.4 M sodium hydroxide (1 L). All insoluble tar was removed by filtration. Concentrated hydrochloric acid (12 M, 70 mL) was added, the resulting brown-orange solid was collected by filtration, washed with water (3X), heptane (2X) and dried at 54°C under vacuum for 6 h. Obtained 34.5 g (208 mmol, 62%) of 6-Chloro-7-methyl-1H-indole-2,3-dione (2).

δ_H (400 MHz, DMSO-d6) 11.3 (1 H, s), 7.4 (1 H, d, *J*=8.0), 7.2 (1 H, d, *J*=8.1), 2.25 (3 H, s).

6-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one (3)

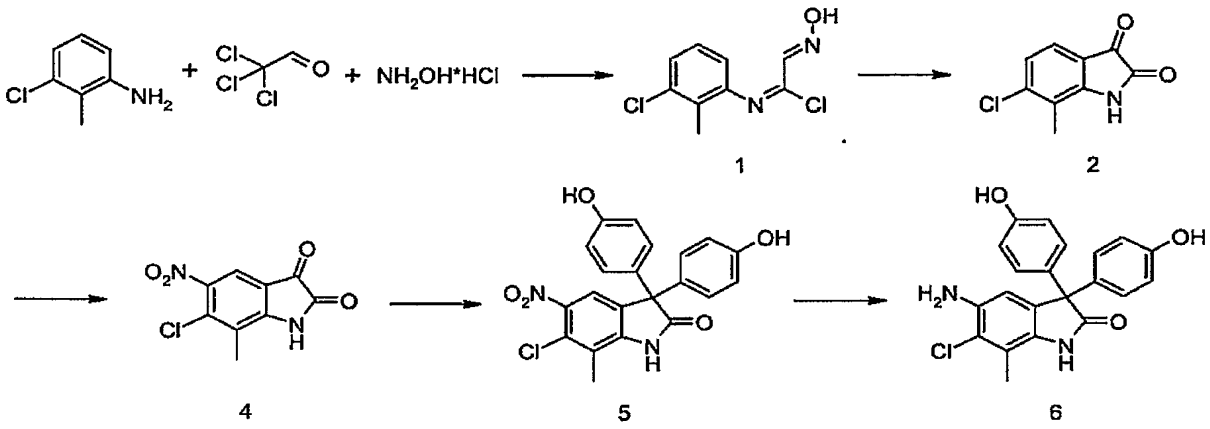


Phenol (15.3 g, 163.6 mmol) and 2 (16.0 g, 81.8 mmol) were suspended in glacial acetic acid (82 ml) and sulphuric acid (18.3 M, 8.8 mL) under nitrogen. The reaction mixture was heated at 85°C, after 2 h left cool to RT, diluted in ethyl acetate and washed with water (3X). The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The crude material was purified by re-crystallization from toluene: ethyl acetate (20 volume: 1 volume) to yield 13.3 g of yellow solid containing sole toluene. Dried overnight in high vacuum at 45°C to yield 10.65 g (29.2 mmol, 38 %) of 6-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one (3) as a white solid.

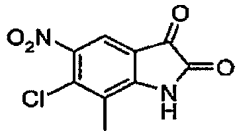
LCMS m/z 366.3 [(Cl^{35}) M+H]$^+$ @ R_T 1.3 min, 100%

δ_H (400 MHz, DMSO-d6) 10.9 (1 H, s), 9.5 (2 H, s), 7.1 (1 H, d, *J*=9.8), 7.05 (1 H, d, *J*=9.6), 6.95 (4 H, d, *J*=10.2), 6.7 (4 H, d, *J*=10.2), 2.35 (3 H, s).

Example 2: Synthetic route followed for 5-Amino-6-chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one (6)



6-Chloro-7-methyl-5-nitro-1H-indole-2,3-dione (4)

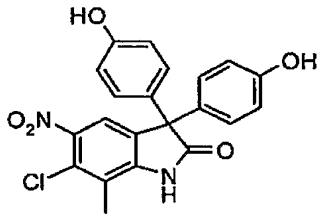


To a well stirred suspension of 2 (2.0 g, 10.2 mmol) in glacial acetic acid (2 mL) and sulphuric acid (4 mL) cooled in ice/water was added cold mixture of nitric acid (69%, 1 g, 10.9 mmol) and sulphuric acid (0.7 g, 7.3 mmol) at such a rate to maintain internal temperature below 5°C. After addition was completed reaction mixture was stirred at RT for 1 h, then slowly poured over ice (~20 g) and left standing for 10 minutes. The solid formed was collected by filtration, washed with cold water (3X), dried under vacuum overnight to yield 1.92 g (8.0 mmol, 78%) of 6-Chloro-7-methyl-5-nitro-1H-indole-2,3-dione (4) as an orange solid.

LCMS m/z 118.79 [Fragment]$^+$ @ R_T 1.14min, 95 %

δ_H (400 MHz, DMSO-d6) 11.7 (1 H, s), 8.1 (1 H, s), 2.3 (3 H, s).

6-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-5-nitro-1,3-dihydro-indol-2-one (5)

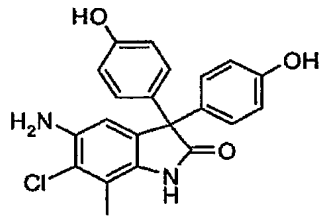


To a suspension of phenol (0.19 g, 2 mmol) and 4 (0.24 g (1 mmol) in glacial acetic acid (1 ml) under nitrogen was added sulphuric acid (18.3 M, 0.2 g, 2 mmol). The mixture was heated at 100°C for 2 h. Crude reaction mixture was neutralised to pH ~7 with 2 M sodium hydroxide, then extracted with ethyl acetate (2X). The organic layer was washed with water (3X), dried (Na_2SO_4) and concentrated under reduced pressure to yield a brown oil. This oil was mixed with DCM to yield a solid that after wash with DCM: AcOEt (99: 1) (3X) gave 0.145 g (0.35 mmol, 35%).

LCMS m/z 411.1 $[(Cl^{35}) M+H]^+$ @ R_T 1.26 min, 93%

δ_H (400 MHz, DMSO-d6) 7.48 (1 H, s), 6.96 – 6.96 (4 H, m), 6.66 – 6.59 (4 H, m), 2.35 (3 H, s).

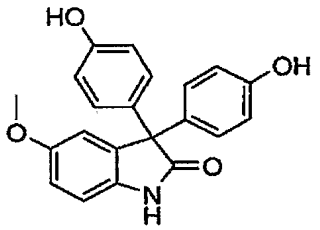
5-Amino-6-chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one (6)



To a solution of 5 (0.1 g, 0.24 mmol) in methanol (2 mL) was added Pd/C (10% w/w, 0.03 g). The black mixture was stirred under hydrogen at RT for 16 h. The catalyst was removed by filtration, and the solvent was removed under reduced pressure to yield 0.084 g (0.22 mmol, 92%) of 5-Amino-6-chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one (6).

LCMS m/z 381.16 [(Cl^{35}) M+H]$^+$ @ R_T 0.94 min, 84%

Example 3: Generic procedure for 3,3-bis-(4-hydroxy-phenyl)-5-methoxy-1,3-dihydro-indol-2-one (7)

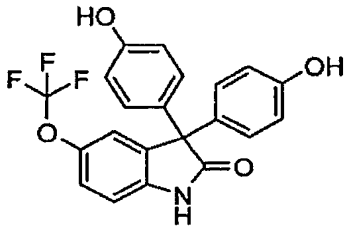


To a suspension of phenol (0.28 g, 2.9 mmol) and 5-methoxy-1H-indole-2,3-dione (0.24 g (1.3 mmol) in glacial acetic acid (1.5 ml) under nitrogen was added sulphuric acid (18.3 M, 0.145 mL). The mixture was heated at 100°C for 2 h. Crude reaction mixture was diluted with water and extracted with ethyl acetate (2X). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to yield a brown solid. This solid was mixed with DCM: AcOEt (9: 1) (3X) and gave 0.08 g (0.35 mmol, 18%) of 3,3-bis-(4-hydroxy-phenyl)-5-methoxy-1,3-dihydro-indol-2-one (7).

LCMS m/z 348.19 [M+H]$^+$ @ R_T 1.09 min, 100%

δ_H (400 MHz, Methanol-d4) 6.92 (4 H, d, *J*=8.80 Hz), 6.79 - 6.82 (1 H, m), 6.69 - 6.73 (1 H, m), 6.61 (5 H, m), 3.62 (3 H, s)

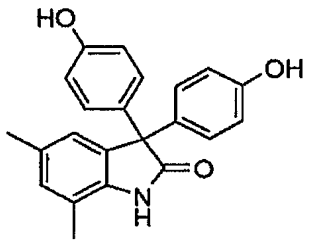
3,3-Bis-(4-hydroxy-phenyl)-5-trifluoromethoxy-1,3-dihydro-indol-2-one (8)



LCMS m/z 402.12 [M+H]$^+$ @ R_T 1.27 min, 96%

δ_H (400 MHz, DMSO-d6) 10.78 (1 H, s), 9.43 (2 H, s), 7.23 (1 H, d, *J*=8.56), 7.17 (1 H, s), 6.99 (1 H, d, *J*=8.56), 6.93 (4 H, d, *J*=8.80), 6.66 (4 H, d, *J*=8.56).

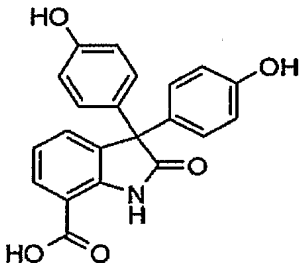
3,3-Bis-(4-hydroxy-phenyl)-5,7-dimethyl-1,3-dihydro-indol-2-one (9)



LCMS m/z 346.19 $[M+H]^+$ @ R_T 1.24 min, 92%

δ_H (400 MHz, DMSO-d6) 10.39 (1 H, s), 9.25 (2 H, s), 6.8 (4 H, d, *J*=8.6), 6.70 (1 H, s), 6.68 (1 H, s), 6.52 (4 H, d, *J*=8.6), 2.09 (6 H, s).

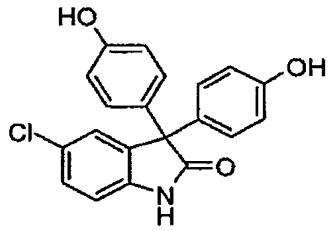
3,3-Bis-(4-hydroxy-phenyl)-2-oxo-2,3-dihydro-1H-indole-7-carboxylic acid (10)



LCMS m/z 362.13 $[M+H]^+$ @ R_T 1.06 min, 90%

δ_H (400 MHz, DMSO-d6) 10.11 (1 H, s), 9.43 (2 H, s), 7.71 (1 H, dd, *J*=8.1, 1.2), 7.38 (1 H, dd, *J*=7.3, 0.7), 7.08 (1 H, t, *J*=7.8), 6.92 (4 H, d, *J*=8.8), 6.67 (4 H, d, *J*=8.8).

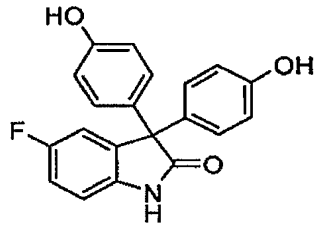
5-Chloro-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-indol-2-one (11)



LCMS m/z 352.11 [(Cl^{35}) M+H]$^+$ @ R_T 1.21 min, 100%

δ_H (400 MHz, DMSO-d6) 10.72 (1 H, s), 9.42 (2 H, s), 7.25 (1 H, dd, *J*=8.2, 2.1), 7.18 (1 H, d, *J*=2.2), 6.89-6.95 (5 H, m), 6.68 (4 H, d, *J*=8.6).

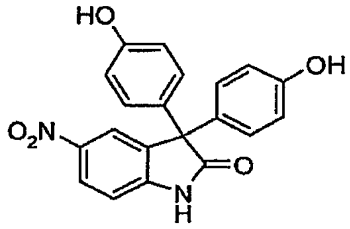
5-Fluoro-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-indol-2-one (12)



LCMS m/z 336.16 [M+H]$^+$ @ R_T 1.14 min, 90%

δ_H (400 MHz, DMSO-d6) 10.61 (1 H, s), 9.41 (2 H, s), 7.00-7.10 (2 H, m), 6.93 (4 H, d, *J*=8.6), 6.89 (1 H, dd, *J*=8.4, 4.5), 6.67 (4 H, d, *J*=8.8).

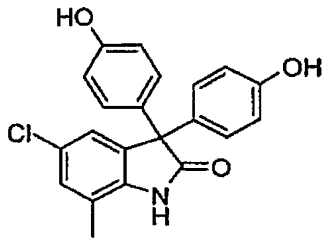
3,3-Bis-(4-hydroxy-phenyl)-5-nitro-1,3-dihydro-indol-2-one (13)



LCMS m/z 362.86 [M+H]$^+$ @ R_T 1.25 min, 93%

δ_H (400 MHz, DMSO-d6) 11.31 (1 H, s), 9.48 (2 H, s), 8.19 (1 H, dd, *J*=8.7, 2.3), 7.90 (1 H, d, *J*=2.2), 7.12 (1 H, d, *J*=8.8), 6.94 (4 H, d, *J*= 8.8), 6.70 (4 H, d, *J*=8.8).

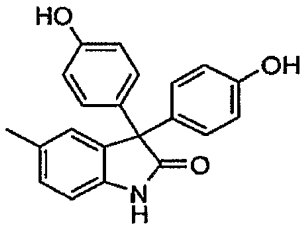
5-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one (14)



LCMS m/z 365.92 [(Cl^{35}) M+H]$^+$ @ R_T 1.36 min, 91%

δ_H (400 MHz, DMSO-d6) 10.77 (1 H, s), 9.41 (2 H, s), 7.10 (1 H, d, *J*=1.5), 6.98 (1 H, d, *J*=1.9), 6.91 (4 H, d, *J*=8.6), 6.67 (4 H, d, *J*=8.6), 2.22 (3 H, s).

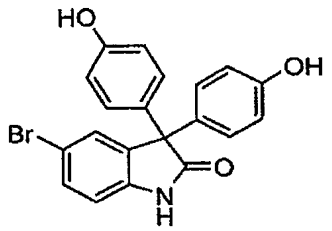
3,3-Bis-(4-hydroxy-phenyl)-5-methyl-1,3-dihydro-indol-2-one (15)



LCMS m/z 331.97 [M+H]$^+$ @ R_T 1.37 min, 91%

δ_H (400 MHz, DMSO-d6) 10.42 (1 H, s), 9.33 (2 H, s), 6.90-6.97 (2 H, m), 6.88 (4 H, d, *J*=8.6), 6.75 (1 H, d, *J*=7.8), 6.62 (4 H, d, *J*=8.8), 2.17 (3 H, s).

5-Bromo-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-indol-2-one (16)



LCMS m/z 396.05 $[(Br^{79})\ M+H]^+$ @ R_T 1.14 min, 94%

δ_H (400 MHz, MeOD) 7.28 (1 H, dd, *J* =8.3, 2.0), 7.14 (1 H, d, *J* =2.0), 6.88-6.92 (4 H, m), 6.81 (1 H, d, *J*=8.3), 6.60-6.64 (4 H, m).

Example 4: Synthesis of Thioamides and Isatins

Thioamides (Q=S and n=1) were obtained by reacting the corresponding amides (Q=O and n=1) with Lawesson's reagent as described in OS Coll. Vol. VII, p372.

All isatins was used in the general procedure described for converting isatins to the final compounds. The isatins used are obtained by either procedure A or procedure B.

Procedure A, based on literature procedures was used to generate aromatic isatins with either electron donating substituents (see Stolle: *J. Prakt. Chem.* (1922), **105**, 137 and Sandmeyer: *Helv. Chim. Acta* (1919), **2**, 234) or a 5-membered electron rich heteroaromatic moiety (see Shvedov et al. (Chem. Heterocycl. Compd. Engl. Transl. (1975), **11**, 666). Examples of preferred 5-membered heterocycles are thiophenes (V^1=S, V^2=V^3=C and V^4=bond; V^2=S, V^1=V^3=C and V^4=bond or V^3=S, V^1=V^2=C and V^4=bond), furans (V^1=O, V^2=V^3=C and V^4=bond; V^2=O, V^1=V^3=C and V^4=bond or V^3=O, V^1=V^2=C and V^4=bond), pyrazoles (V^1=NR, V^2=N, V^3=C and V^4=bond; V^1=N, V^2=NR, V^3=C and V^4=bond) and imidazoles (V^1=NR, V^2=C, V^3=N and V^4=bond; V^1=N, V^2=C, V^3=NR and V^4=bond).

Procedure B, based on literature procedures was used to generate aromatic isatins with electron withdrawing substituents (see Hewawasam and Maenwell: *Tet. Lett.* (1994), **35**, 7303) and 6-membered electron poor heteroaromatic isatins (see Rivalle and Bisagni: *J. Heterocycl. Chem.* (1997), **34**, 441). Examples of preferred 6-membered heterocycles are pyridines (V^1=N, V^2=V^3=V^4=C; V^2=N, V^1=V^3=V^4=C; V^3=N, V^1=V^2=V^4=C and V^4=N,

$V^1=V^2=V^3=C$), pyrimidines ($V^1=V^3=N$, $V^2=V^4=C$; $V^2=V^4=N$, $V^1=V^3=C$), pyrazines ($V^1=V^4=N$, $V^2=V^3=C$) and pyridazines ($V^1=V^2=N$, $V^3=V^4=C$; $V^2=V^3=N$, $V^1=V^4=C$; $V^3=V^4=N$, $V^1=V^2=C$).

Example 5: Cell proliferation

Inhibition of the proliferation of human cancer cells is widely used to predict the anti-cancer potential of novel chemicals. Typically, human cancer cell lines derived from tumour material are maintained in monolayer cultures and test chemicals are added for varying durations. Test compounds with anti-cancer potential are expected to reduce proliferation and thereby reduce cell number relative to vehicle treated control cell cultures. Cell number can be monitored by cell counting, determining metabolic rate (e.g.metabolic reduction of tetrazolium salts such as (3-(4,5-dimethylethiazol-2-yl)-2,5-diphenyltetrazolium bromide or alamarBlue), quantifying DNA content (using DNA binding dyes such as BODIPY-FL-14-dUTP) or measuring nucletotide incorporation into DNA (e.g. radiolabelled thymidine or bromo-deoxyuridine incorporation).

One important consideration is whether any inhibitory effects of test compounds are specific to cancer cell proliferation or are due to general inhibition of cell proliferation. This issue can be addressed using paired cell lines; for example, the effects of test compounds on the proliferation of transformed cancer cell lines can be compared with the effects of test compounds on the proliferation of untransformed cells from the same tissue source. Alternatively, phenotypic differences between cancer cell lines can be exploited to evaluate the selectivity of test compounds. For example, the anti-proliferative effects of some compounds are only apparent in certain sub-types of human breast cancer cell lines (e.g. breast cancer cell lines cell lines with PTEN gene mutations or gene amplification of the p70S6K protein kinase), but not in breast cancer line lines that do not exhibit this phenotype (Noh et al (2004) Clinical Cancer Research 10, 1013-1023; Yu et al (2001) Endocrine-Related Cancer 8, 249-258). The selectivity of test compounds in the latter models is associated with the mechanism of compound action and is related to the presence, absence or relative abundance of the protein target of the test compound in the relevant cell lines.

Method

Compound effects were evaluated on the proliferation of MDA-468 and MDA-231 human breast cancer cells. Cells were maintained in growth medium : RPMI 1640 containing 10% foetal bovine serum and 1% pen/strep. Cells were split 1:4 or 1:8 twice a week when 90%

confluent. For the cell proliferation assay, cells were plated at 8000 cell/well into 96 well black Packard Viewplates in growth medium. After 1 day, the growth medium was replaced with growth medium containing test compounds or vehicle, and cells were maintained in culture for a further 2 days. Growth medium was then removed and replaced with 150µl of alamarBlue in RPMI medium containing 1% pen/strep. Following a 120 minute incubation at 37°C, fluorescent intensity was read using a plate reader.

Results

The results shown in Figure 1, Figure 2, and Figure 3 demonstrate the ability of the majority of the compounds of the general formula (I) inhibit the proliferation of MDA468 human breast cancer cells at lower concentrations as those required to inhibit proliferation of MDA231 human breast cancer cells.

Based on the biological data obtained so far, it is believe that the group R^1 has limited importance for the biological effect, and that it suitably should be a fairly "small", e.g. methyl, ethyl, iso-propyl, methoxy, ethoxy, iso-propoxy, etc.

The group R^2 apparently plays a more important role and may suitably be selected from hydrogen, chloro, phenyl, phenoxy, optionally substituted thiophen-2-yl, and optionally substituted thiophen-3-yl. The preliminary results indicate that a substituent like chloro or a substituent occupying about the space will be particularly relevant.

R^3 is typically selected from hydrogen, methoxy, fluoro, chloro, cyano, phenyl, phenoxy, optionally substituted thiophen-2-yl, and optionally substituted thiophen-3-yl, amino, acetylamino, methylsulfonylamino, and dimethylaminosulfonyl. The group R^3 is typically included in order to avoid bioreactions which may lead to degradation or inactivation of the compound.

The groups X^1 and X^2 are (independently) typically are selected from hydroxy, OAc, NH_2, NMe_2, NHAc, $NHSO_2Me$ and $NHCONMe_2$. For the following reasons, X^1 and X^2 are preferably the same for both phenyl rings. This has the advantage that achiral compounds are achieved. In the pharmaceutical business, use of chiral drugs typically requires isolation of the individual stereoisomeric forms. Another advantage is seen in the synthesis route. A one-step introduction of the two PhX groups saves at least one synthesis step and associated time, and increases the overall yield.

Example 6: Protein synthesis studies

The purpose of these studies as to investigate compounds of the general formula (I) have effect on protein synthesis, measured as ^{14}C-Leucine uptake or incorporation into proteins. As described in "Leucine Uptake [14C] Cytostar-T assay, Amersham Biosciences" (CFA773).

MDA-MB-231 and -468 were seeded at 8000 cells/well in CytoStar-T 96-well microplates. And incubated overnight in growth medium. The next day medium was carefully aspirated (8-channel Vacuboy) and 50 µL of fresh pre-warmed medium (10% FCS, 10 mM HEPES pH 7.2 – 7.5) was added. Cells were allowed to equilibrate at 37 °C for 60 min. Test compounds were added in 50 µL medium and 14C-Leucine was added in 100 µL medium (0.5 µCi mL-1 final). Plates sealed with transparent, adhesive foil. Plates were then incubated in a 37 °C for 6h in a humidified incubator. Incorporation of radioactive leucine into proteins (a measure of protein synthesis) was then read by coincidence scintillation (counts per minute (CPM)) using a Wallac Microbeta detector at the indicated time-intervals. A reading a t=0 (5 min after sealing plates) for each well is subtracted as background.

The results are shown in Figure 4 measured after 6hours.

The results indicates that BIC0043901 significantly inhibits 14C-Leucine incorporation in MDA-MB-468 in a concentration dependent manner observed after 240 min compound incubation and up to 22 hours. EC50 is estimated to 100 nM (240 min to 22 hours). Interestingly, the effect seems to reach a plateau at the high concentrations corresponding to approx. 1/6 of total incorporated. This indicates that there is some proportion of the protein synthesis that BIC0043901 is not able to affect.

No significant effect of BIC0043901 was observed in MDA-MB-231 up to 430 min. At 22 hours a minor effect is observed at 30 µM. EC50 >> 30 µM (22 hours).

The inhibitory effect of BIC0043901 is therefore very specific for MDA-MB-468.

The higher concentrations of the control compounds Anisomycin and Cycloheximide completely inhibits incorporation at all time-points (as opposed to BIC0043901, se above).

Example 7: Western Blot Studies

To investigate the mechanism of action of compounds of general formula (I) Western Blot studies were performed to investigate the activation state of pathways linked to the regulation of protein synthesis (see Figure 5).

Method

MDA-MB-468 cells (also called MDA468) or MDA-MB-231 (also called MDA231) were kept in culture and plated at 400 000 cells/well in 6 well cell culture plate. 16-24 hours after the growth medium were shifted to growth medium containing compounds.

After 24 or 48 hours incubation with compounds cells were washed with ice cold PBS buffer and harvested in lysis buffer: Cytobuster reagent (Novagen) containing phosphatase inhibitor cocktail 1 and 2 and protease inhibitor cocktail (Sigma). Samples containing an equal amount of protein were loaded onto 7% Tris Acetate gels, 10% Bis-Tris in MES buffer or 12% Bis-Tris gels using MOPS running buffer (Invitrogen). Following electrophoresis the samples were blotted onto a PVDF membrane (Invitrogen). For membrane blocking and antibody incubations of p70 S6K, Phospho-p70 S6K (Thr389), Pathscanl and S6 antibodies (Cell Signalling Technology) a buffer containing 0.2% Tween-20, 5% non fat dry milk, 5% FBS, in Tris buffered Saline (TBS) were used. For immunoblotting of 4EBP1, Phospho 4EBP1 (Thr37/46), Phospho 4EBP1 (Ser65) (Cell Signalling Technology) and Cyclin D3 (Santa Cruz) a protocol from Cell Signalling Technology were used. Cell Signalling Technology blocking buffer contains 0.1% Tween-20, 5% non fat dry milk in TBS and primary antibody dilution buffer contains 0.1% Tween-20, 5% BSA in TBS. Before adding primary antibody dilution buffer to the membranes the blots were rinsed briefly in 0.1% Tween-20. All antibody incubations were done overnight at 4°C overnight. After washing the membranes with 0.1% Tween-20 in TBS, the blots were incubated with horseradish peroxidase conjugated anti-Rabbit IgG (1:1000-1:3000; Amersham Biosciences) at room temperature for 1h. Peroxidase activity was detected using the ECL detection system (Amersham Biosciences).

Results

Western blot analyses demonstrate that compounds of general formula (I), such as BIC0043901, inhibit the phosphorylation of p70S6K and S6 ribosomal protein in MDA468 cells following 24 hour incubation (Figure 6). Similar effects are observed with the mTOR inhibitor, rapamycin and the PI3 kinase inhibitor LY294002. AKT phosphorylation on Ser473 is not inhibited by BIC0043901 or rapamycin, whereas LY294002 inhibits the phosphorylation of AKT on Ser473. Furthermore, BIC0043901 induces a gel mobility shift in 4E-BP1 as shown using both total and thr37/46 phospho-specific anti-4E-BP1 antibodies, indicative of an alteration in the phosphorylation status of 4E-BP1. This is confirmed by the inhibitory effect of BIC0043901 on the phosphorylation of ser65 of 4E-BP1. Similar effects are observed with the mTOR inhibitor, rapamycin and the PI3 kinase inhibitor LY294002. In addition, expression of the cell cycle regulatory protein cyclin D3 is reduced by BIC0043901, rapamycin and LY294002. These data suggest that mammalian homologue of TOR (mTOR) kinase is active in MDA468 cells under growth conditions, leading to phosphorylation of mTOR target proteins such as p70S6 kinase (p70S6K) and 4EBP1, and downstream regulation of protein synthesis and cell proliferation via S6 ribosomal protein, eukaryotic translation initiation factor, eIF4, and cyclin D3. Compounds of general formula (I), such as BIC0043901, as well as rapamycin and LY294002, inhibit this pathway in MDA468 cells and might be expected to reduce protein synthesis and cell proliferation.

Compounds of general formula (I) such as BIC0043901 did not inhibit the phosphorylation of p70S6K, or induce a gel mobility shift in total p70S6K, in MDA231 cells following 48 hour incubation (Figure 7). In contrast, rapamycin and LY294002 inhibit the phosphorylation of p70S6K, and induce a gel mobility shift in total p70S6K, following 48 hour incubation in MDA231 cells. BIC0043901, rapamycin and LY294002 all inhibit the phosphorylation of p70S6K and induce a gel mobility shift in total p70S6K in MDA468 cells following 48 hour incubation, demonstrating a cell selective effect of compounds of general formula (I), such as BIC0043901.

Example 8: Xenograft studies

The purpose of this study was to evaluate whether compounds of general formula (I), such as BIC0043901, inhibit the growth of cancer cells in a xenograft animal model.

Method

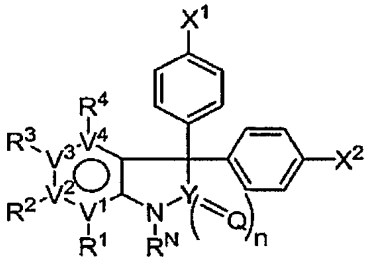
Male nude NMRU nu/nu mice weighing 25-45 grams are implanted with PRXF PC3M tumours by subcutaneous implantation in both flanks. BIC0043901/SCR0044001 (50 & 100mg) is administered daily by the per-oral (PO) route in an appropriate vehicle (2% DMSO:5% Tween 80: 93% saline) either alone or in combination with a sub-optimal dose of paclitaxol (10mg/kg ; intravenous; given once/week). Tumor diameter is determined twice/week for a period of 14 days.

Results

BIC0043901/SCR0044001 reduces the rate of tumour cell growth when given as a monothewrapy (see Figure 8). Furthermore, additive anti-growth effects are noted in combination with paclitaxol.

CLAIMS

1. Use of a compound of the general formula (I)



(I)

wherein

V^1, V^2, V^3, and V^4 independently are selected from a carbon atom, a non-quaternary nitrogen atom, an oxygen atom, and a sulfur atom, and where V^4 further may be selected from a bond, so that $-V^1-V^2-V^3-V^4-$ together with the atoms to which V^1 and V^4 are attached form an aromatic or heteroaromatic ring;

R^1, R^2, R^3, and R^4, when attached to a carbon atom, independently are selected from hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, hydroxy, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{2-6}-alkenyloxy, carboxy, optionally substituted C_{1-6}-alkoxycarbonyl, optionally substituted C_{1-6}-alkylcarbonyl, optionally substituted C_{1-6}-alkylcarbonyloxy, formyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylsulphonylamino, cyano, carbamido, mono- and di(C_{1-6}-alkyl)aminocarbonylamino, C_{1-6}-alkanoyloxy, C_{1-6}-alkylsulphonyl, C_{1-6}-alkylsulphinyl, aminosulfonyl, mono- and di(C_{1-6}-alkyl)aminosulfonyl, nitro, optionally substituted C_{1-6}-alkylthio, and halogen, where any C_{1-6}-alkyl as an amino substituent is optionally substituted with hydroxy, C_{1-6}-alkoxy, amino, mono- and di(C_{1-6}-alkyl)amino, carboxy, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylaminocarbonyl, or halogen(s);

R^1, R^2, R^3, and R^4, when attached to a nitrogen atom, independently are selected from hydrogen, optionally substituted C_{1-6}-alkyl, hydroxy, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkoxycarbonyl, optionally substituted C_{1-6}-alkylcarbonyl, formyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino, C_{1-6}-alkylcarbonylamino, mono- and di(C_{1-6}-alkyl)amino, C_{1-6}-alkylsulphonyl, and C_{1-6}-alkylsulphinyl; where any C_{1-6}-alkyl as an amino substituent is optionally substituted with hydroxy, C_{1-6}-alkoxy, amino, mono- and di(C_{1-6}-alkyl)amino, carboxy, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylaminocarbonyl, or halogen(s);

or R^1 and R^2 together with the carbon atoms to which they are attached form a heterocyclic or heteroaromatic ring;

provided that R^1, R^2, R^3 and R^4 are not all hydrogen;

X^1 and X^2 are independently selected from hydroxy, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkylcarbonyloxy, amino, mono- and di(C_{1-6}-alkyl)amino, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylsulphonylamino, mono- and di(C_{1-6}-alkyl)aminocarbonylamino, C_{1-6}-alkanoyloxy, and mono- and di(C_{1-6}-alkyl)aminosulfonyl, where any C_{1-6}-alkyl as an amino substituent is optionally substituted with hydroxy, C_{1-6}-alkoxy, amino, mono- and di(C_{1-6}-alkyl)amino, carboxy, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylaminocarbonyl, or halogen(s);

$Y(=Q)_n$ is selected from >C=O, >C=S, >S=O and $>S(=O)_2$; and

R^N is selected from the group consisting of hydrogen, optionally substituted C_{1-6}-alkyl, hydroxy, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkoxycarbonyl, optionally substituted C_{1-6}-alkylcarbonyl, formyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino, C_{1-6}-alkylcarbonyamino, mono- and di(C_{1-6}-alkyl)amino, C_{1-6}-alkylsulphonyl, and C_{1-6}-alkylsulphinyl; where any C_{1-6}-alkyl as an amino substituent is optionally substituted with hydroxy, C_{1-6}-alkoxy, amino, mono- and di(C_{1-6}-alkyl)amino, carboxy, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylaminocarbonyl, or halogen(s); and

pharmaceutically acceptable salts and prodrugs thereof;

for the preparation of a medicament for the treatment of cancer in a mammal.

2. The use according to claim 1, wherein $-V^1-V^2-V^3-V^4-$ together with the atoms to which V^1 and V^4 are attached form a ring selected from a benzene ring, a pyridine ring, a thiophene ring (V^1=S, V^2=V^3=C and V^4=bond; V^2=S, V^1=V^3=C and V^4=bond; or V^3=S, V^1=V^2=C and V^4=bond), a furan ring (V^1=O, V^2=V^3=C and V^4=bond; V^2=O, V^1=V^3=C and V^4=bond; or V^3=O, V^1=V^2=C and V^4=bond), a pyrazole ring (V^1=NR, V^2=N, V^3=C and V^4=bond; V^1=N, V^2=NR, V^3=C and V^4=bond), an imidazole ring (V^1=NR, V^2=C, V^3=N and V^4=bond; V^1=N, V^2=C, V^3=NR and V^4=bond), a pyridine ring (V^1=N, V^2=V^3=V^4=C; V^2=N, V^1=V^3=V^4=C; V^3=N, V^1=V^2=V^4=C and V^4=N, V^1=V^2=V^3=C), a pyrimidine ring (V^1=V^3=N, V^2=V^4=C; V^2=V^4=N, V^1=V^3=C), pyrazines (V^1=V^4=N, V^2=V^3=C), and a pyridazine ring (V^1=V^2=N, V^3=V^4=C; V^2=V^3=N, V^1=V^4=C; V^3=V^4=N, V^1=V^2=C).

3. The use according to claim 2, wherein the ring is selected from a benzene ring and a pyridine ring where the nitrogen atom represents V^3.

4. The use according to any one of the claims 1-3, wherein R^1, R^2, R^3, and R^4 independently are selected from hydrogen, optionally substituted C_{1-6}-alkyl, hydroxy, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkoxycarbonyl, optionally substituted C_{1-6}-alkylcarbonyl, amino, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylsulphonylamino, mono- and di(C_{1-6}-alkyl)aminosulfonyl, and mono- and di(C_{1-6}-alkyl)amino, where any C_{1-6}-alkyl as an amino substituent is optionally substituted with hydroxy, C_{1-6}-alkoxy, amino, mono- and di(C_{1-6}-alkyl)amino, carboxy, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylaminocarbonyl, or halogen(s).

5. The use according to any one of the claims 1-4, wherein R^1 is selected from hydrogen, halogen, C_{1-6}-alkyl, trifluoromethyl and C_{1-6}-alkoxy, when V^1 is a carbon atom.

6. The use according to any one of the claims 1-5, wherein R^2 is selected from hydrogen, halogen, optionally substituted aryl, optionally substituted aryloxy, and optionally substituted heteroaryl, when V^2 is a carbon atom.

7. The use according to any one of the claims 1-6, wherein R^3 is selected from hydrogen, optionally substituted C_{1-6}-alkoxy, halogen, cyano, optionally substituted aryl, optionally substituted aryloxy, optionally substituted heteroaryl, amino, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylsulphonylamino, and mono- and di(C_{1-6}-alkyl)aminosulfonyl, when V^3 is a carbon atom.

8. The use according to any one of the claims 1-7, wherein R^4 is hydrogen, when V^4 is a carbon atom.

9. The use according to any one of the claims 1-8, wherein X^1 and X^2 independently are selected from OR^6, $OCOR^5$, $N(R^6)_2$, $NHCOR^5$, $NHSO_2R^5$, and $NHCON(R^6)_2$, wherein R^5 is selected from C_{1-6}-alkyl, optionally substituted aryl and optionally substituted heteroaryl, and each R^6 independently is selected from hydrogen, C_{1-6}-alkyl, optionally substituted aryl and optionally substituted heteroaryl.

10. The use according to any one of the claims 1-9, wherein X^1 and X^2 independently are selected from hydroxy, OAc, NH_2, NMe_2, NHAc, $NHSO_2Me$ and $NHCONMe_2$.

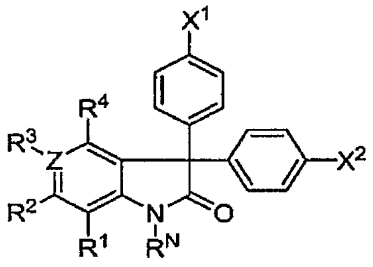
11. The use according to any one of the claims 1-10, wherein X^1 and X^2 are the same.

12. The use according to any one of the claims 1-11, wherein Y is a carbon atom and Q is an oxygen atom.

13. The use according to any one of the claims 1-11, wherein Y is a sulfur atom, n is 2, and each Q is an oxygen atom.

14. The use according to any one of the claims 1-13, wherein R^N is selected from hydrogen, C_{1-6}-alkyl, amino, and C_{1-6}-alkylcarbonylamino.

15. Use of a 3,3-Diphenyl-1,3-dihydro-indol-2-one type compound of the formula (II)



(II)

wherein

R^1 is selected from hydrogen, halogen, C_{1-6}-alkyl, trifluoromethyl and C_{1-6}-alkoxy;

R^2 is selected from hydrogen, halogen, optionally substituted aryl, optionally substituted aryloxy, and optionally substituted heteroaryl;

R^3 is selected from hydrogen, optionally substituted C_{1-6}-alkoxy, halogen, cyano, and optionally substituted aryl, optionally substituted aryloxy, optionally substituted heteroaryl, amino, C_{1-6}-alkylcarbonylamino, C_{1-6}-alkylsulphonylamino, and mono- and di(C_{1-6}-alkyl)aminosulfonyl,;

R^4 and R^N are both hydrogen;

Z is CH or N; and

X^1 and X^2 are independently selected from OR^6, $OCOR^5$, $N(R^6)_2$, $NHCOR^5$, $NHSO_2R^5$, and $NHCON(R^6)_2$, wherein R^5 is selected from C_{1-6}-alkyl, optionally substituted aryl and optionally substituted heteroaryl, and each R^6 independently is selected from hydrogen, C_{1-6}-alkyl, optionally substituted aryl and optionally substituted heteroaryl; and

pharmaceutically acceptable salts and prodrugs thereof;

for the preparation of a medicament for the treatment of cancer in a mammal.

16. The use according to claim 15, wherein R^1 is selected from C_{1-6}-alkyl and C_{1-6}-alkoxy, such as from methyl, ethyl, isopropyl, methoxy, ethoxy and isopropoxy, in particular from methoxy, ethoxy and isopropoxy, or from methyl, ethyl, and isopropyl.

17. The use according to any one of claims 15-16, wherein R^2 is selected from hydrogen, chloro, phenyl, phenoxy, optionally substituted thiophen-2-yl, and optionally substituted thiophen-3-yl.

18. The use according to any one of claims 15-17, wherein R^3 is selected from hydrogen, methoxy, fluoro, chloro, cyano, phenyl, phenoxy, optionally substituted thiophen-2-yl, and optionally substituted thiophen-3-yl, amino, acetylamino, methylsulfonylamino, and dimethylaminosulfonyl.

19. The use according to any one of claims 15-18, wherein X^1 and X^2 independently are selected from hydroxy, OAc, NH_2, NMe_2, NHAc, $NHSO_2Me$ and $NHCONMe_2$.

20. The use according to any one of claims 15-19, wherein X^1 and X^2 are the same.

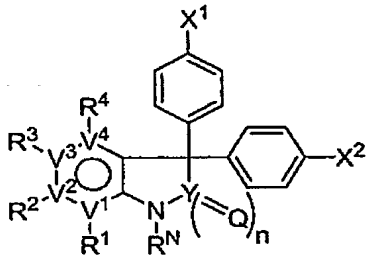
21. The use according to any one of claims 15-20, wherein the compound is selected from:

5-Amino-6-chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one;
5-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one;
5-Fluoro-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-indol-2-one;
3,3-Bis-(4-hydroxy-phenyl)-5-nitro-1,3-dihydro-indol-2-one;
3,3-Bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one;
6-Bromo-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one;
6-Bromo-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one;
6-Bromo-3,3-bis-(4-hydroxy-phenyl)-5,7-dimethyl-1,3-dihydro-indol-2-one;
6-Bromo-3,3-bis-(4-hydroxy-phenyl)-7-methyl-2-oxo-2,3-dihydro-1H-indole-5-carbonitrile;
6-Bromo-3,3-bis-(4-hydroxy-phenyl)-5-methoxy-7-methyl-1,3-dihydro-indol-2-one;
6-Bromo-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one;
6-Bromo-7-ethyl-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one;
6-Bromo-7-ethyl-3,3-bis-(4-hydroxy-phenyl)-5-methyl-1,3-dihydro-indol-2-one;
6-Bromo-5-ethyl-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one;
6-Bromo-7-ethyl-3,3-bis-(4-hydroxy-phenyl)-2-oxo-2,3-dihydro-1H-indole-5-carbonitrile;
6-Bromo-7-ethyl-3,3-bis-(4-hydroxy-phenyl)-5-methoxy-1,3-dihydro-indol-2-one;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-5,7-dimethyl-1,3-dihydro-indol-2-one;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-2-oxo-2,3-dihydro-1H-indole-5-carbonitrile;

6-Chloro-3,3-bis-(4-hydroxy-phenyl)-5-methoxy-7-methyl-1,3-dihydro-indol-2-one;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one;
6-Chloro-7-ethyl-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one;
6-Chloro-7-ethyl-3,3-bis-(4-hydroxy-phenyl)-5-methyl-1,3-dihydro-indol-2-one;
6-Chloro-5-ethyl-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one;
6-Chloro-7-ethyl-3,3-bis-(4-hydroxy-phenyl)-2-oxo-2,3-dihydro-1H-indole-5-carbonitrile;
6-Chloro-7-ethyl-3,3-bis-(4-hydroxy-phenyl)-5-methoxy-1,3-dihydro-indol-2-one;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methoxy-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-5-methyl-7-methoxy-1,3-dihydro-indol-2-one;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methoxy-2-oxo-2,3-dihydro-1H-indole-5-carbonitrile;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methoxy-1,3-dihydro-pyrrolo[3,2-c]pyridin-2-one;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methoxy-5-methyl-1,3-dihydro-indol-2-one;
6-Chloro-5-ethyl-3,3-bis-(4-hydroxy-phenyl)-7-methoxy-1,3-dihydro-indol-2-one;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-5,7-dimethoxy-1,3-dihydro-indol-2-one;
N-{4-[3-(4-Acetylamino-phenyl)-5-chloro-7-methyl-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl}-acetamide;
N-{4-[5-Chloro-3-(4-methanesulfonylamino-phenyl)-7-methyl-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl}-methanesulfonamideN-{4-[3-(4-Acetylamino-phenyl)-6-chloro-7-methyl-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl}-acetamide;
N-{4-[6-Chloro-3-(4-methanesulfonylamino-phenyl)-7-methyl-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl}-methanesulfonamide;
N-{4-[3-(4-Acetylamino-phenyl)-5-chloro-7-methoxy-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl}-acetamide;
N-{4-[5-Chloro-3-(4-methanesulfonylamino-phenyl)-7-methoxy-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl}-methanesulfonamide;
N-{4-[3-(4-Acetylamino-phenyl)-6-chloro-7-methoxy-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl}-acetamide; and
N-{4-[6-Chloro-3-(4-methanesulfonylamino-phenyl)-7-methoxy-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl}-methanesulfonamide;.

22. A compound as defined in any one of the claims 1-21 for use as a medicament, with the proviso that the compound is not one selected from 3,3-Bis-(4-hydroxy-phenyl)-1,3-dihydro-indol-2-one and acetic acid 4-[3-(4-acetoxy-phenyl)-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl ester.

23. A compound of the general formula (I)



(I)

as defined in any one of the claims 1-14, with the proviso that the compound is not one selected from

3,3-Bis-(4-hydroxy-phenyl)-1,3-dihydro-indol-2-one,

3,3-Bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one;

3,3-Bis-(4-hydroxy-phenyl)-4,5-dimethyl-1,3-dihydro-indol-2-one ;

3,3-Bis-(4-hydroxy-phenyl)-5,7-dimethyl-1,3-dihydro-indol-2-one;

5-Bromo-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-indol-2-one;

5-Chloro-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-indol-2-one;

3,3-Bis-(4-hydroxy-phenyl)-5-methoxy-1,3-dihydro-indol-2-one;

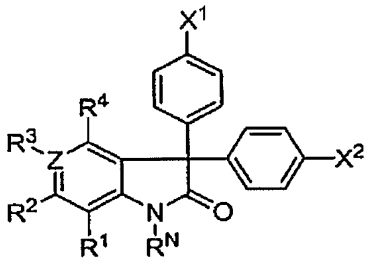
3,3-Bis-(4-hydroxy-phenyl)-5-methyl-1,3-dihydro-indol-2-one;

6-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one;

Acetic acid 4-[3-(4-acetoxy-phenyl)-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl ester; and

Acetic acid 4-[3-(4-acetoxy-phenyl)-5-methyl-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl ester.

24. A 3,3-Diphenyl-1,3-dihydro-indol-2-one type compound of the formula (II)



(II)

as defined in any one of the claims 15-21, with the proviso that the compound is not one selected from:

- 3,3-Bis-(4-hydroxy-phenyl)-1,3-dihydro-indol-2-one,
- 3,3-Bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one;
- 3,3-Bis-(4-hydroxy-phenyl)-4,5-dimethyl-1,3-dihydro-indol-2-one ;
- 3,3-Bis-(4-hydroxy-phenyl)-5,7-dimethyl-1,3-dihydro-indol-2-one;
- 5-Bromo-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-indol-2-one;
- 5-Chloro-3,3-bis-(4-hydroxy-phenyl)-1,3-dihydro-indol-2-one;

3,3-Bis-(4-hydroxy-phenyl)-5-methoxy-1,3-dihydro-indol-2-one;
3,3-Bis-(4-hydroxy-phenyl)-5-methyl-1,3-dihydro-indol-2-one;
6-Chloro-3,3-bis-(4-hydroxy-phenyl)-7-methyl-1,3-dihydro-indol-2-one;
Acetic acid 4-[3-(4-acetoxy-phenyl)-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl ester; and
Acetic acid 4-[3-(4-acetoxy-phenyl)-5-methyl-2-oxo-2,3-dihydro-1H-indol-3-yl]-phenyl ester.

25. A pharmaceutical composition comprising a compound as defined in any one of the claims 1-21 and a pharmaceutically acceptable carrier.

26. The pharmaceutical composition according to claim 25, which is in unit dosage form.

27. The pharmaceutical composition according to claim 26, wherein the each unit dosage form comprises 0.1-50 mg of the compound.

28. The pharmaceutical composition according to any one of the claim 25-27, wherein the compound is as defined in claim 22.

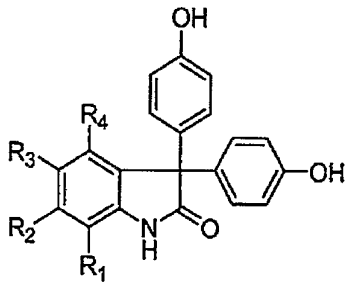
29. The pharmaceutical composition according to any one of the claim 25-27, wherein the compound is as defined in claim 23.

30. The pharmaceutical composition according to any one of the claim 25-27, wherein the compound is as defined in claim 24.

31. A method of treating a mammal suffering from or being susceptible to cancer, the method comprising administering to the mammal a therapeutically effective amount of a compound according to any of the claims 1-21.

32. Measurement of either p70S6K, 4E-BP1 or S6K phosphorylation status using phosphor-specific or total protein antibodies by Western blot or ELISA, or measurement of p70S6K kinase activity, in tumour material or blood samples, for patient selection, or confirmation of compound efficacy in human patients.

Figure 1



BIC No	MDA-231	MDA-468	R1	R2	R3	R4
BIC0043901	7,8	0,009	Me	Cl	H	H
BIC0335322	>40	0,313	Me	Cl	NH2	H
BIC0335323	10,3	8,4	Me	Cl	NO2	H
BIC0335289	6,7	0,044	Me	H	Cl	H
BIC0335134	12,4	0,162	Me	H	Me	H
BIC0334554	>40	0,253	H	H	H	H
BIC0335287	13,3	0,192	H	H	Me	H
BIC0335293	>40	0,18	H	H	OMe	H
BIC0335294	12,7	0,171	H	H	F	H
BIC0335295	12,1	0,095	H	H	Cl	H
BIC0289557	12,3	0,075	H	H	Br	H
BIC0335304	12,8	0,065	H	H	NO2	H
BIC0335133	9,8	2,6	H	H	OCF3	H

Figure 2

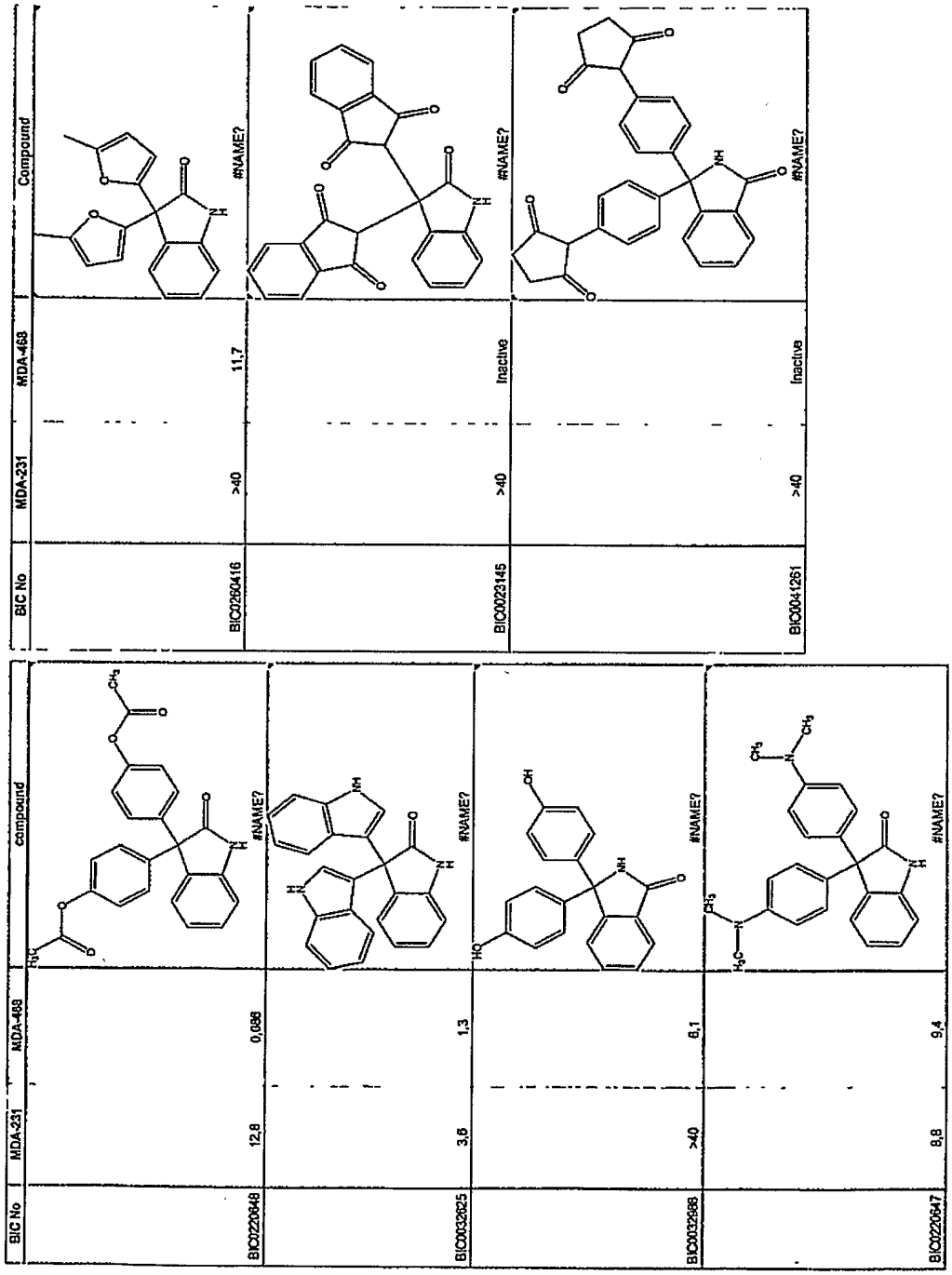
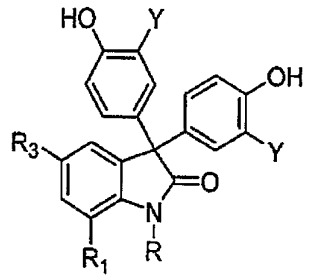


Figure 3



BIC No	MDA-231	MDA-468	R	R1	R3
BIC0292210	8,3	9,2	H	H	Et
BIC0297539	7,7	7,1	H	Me	NO2
BIC0043915	11,3	10,7	H	Br	Br
BIC0335009	13	12,5	H	Me	Br
BIC0335072	13,9	12,9	H	Br	Me
BIC0335073	13,4	13,3	H	H	I
BIC0102531	Inactive	Inactive	H	Me	NO2
BIC0335007	9,6	9	Me	H	Br

Figure 4

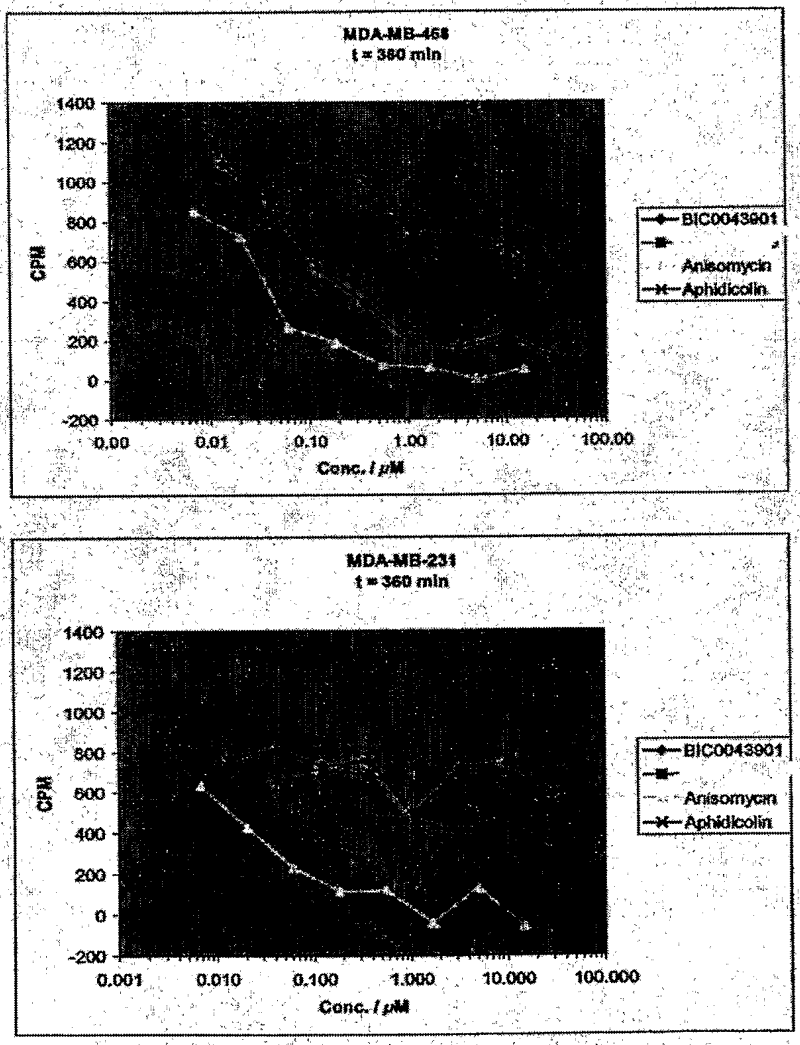


Figure 5

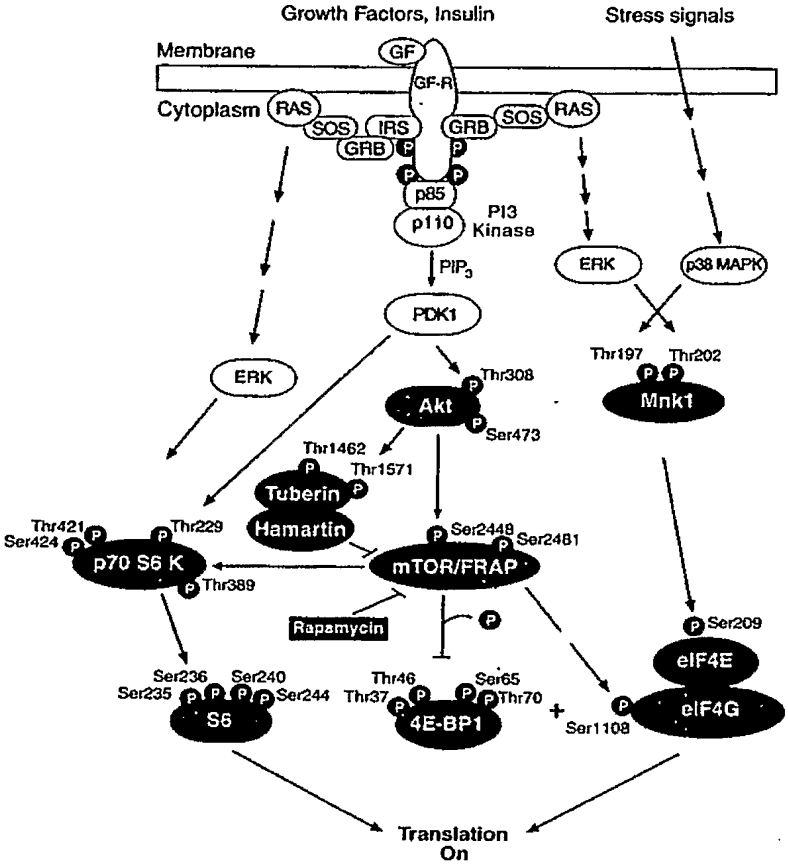


FIGURE 6: MDA468 Cells (24 hour compound incubation)

1: DMSO	0,08%
2: BIC0043901	200 nM
3: BIC0043901	2 μM
4: other	2 μM
5: Rapamycin	100 nM
6: LY294002	10 μM

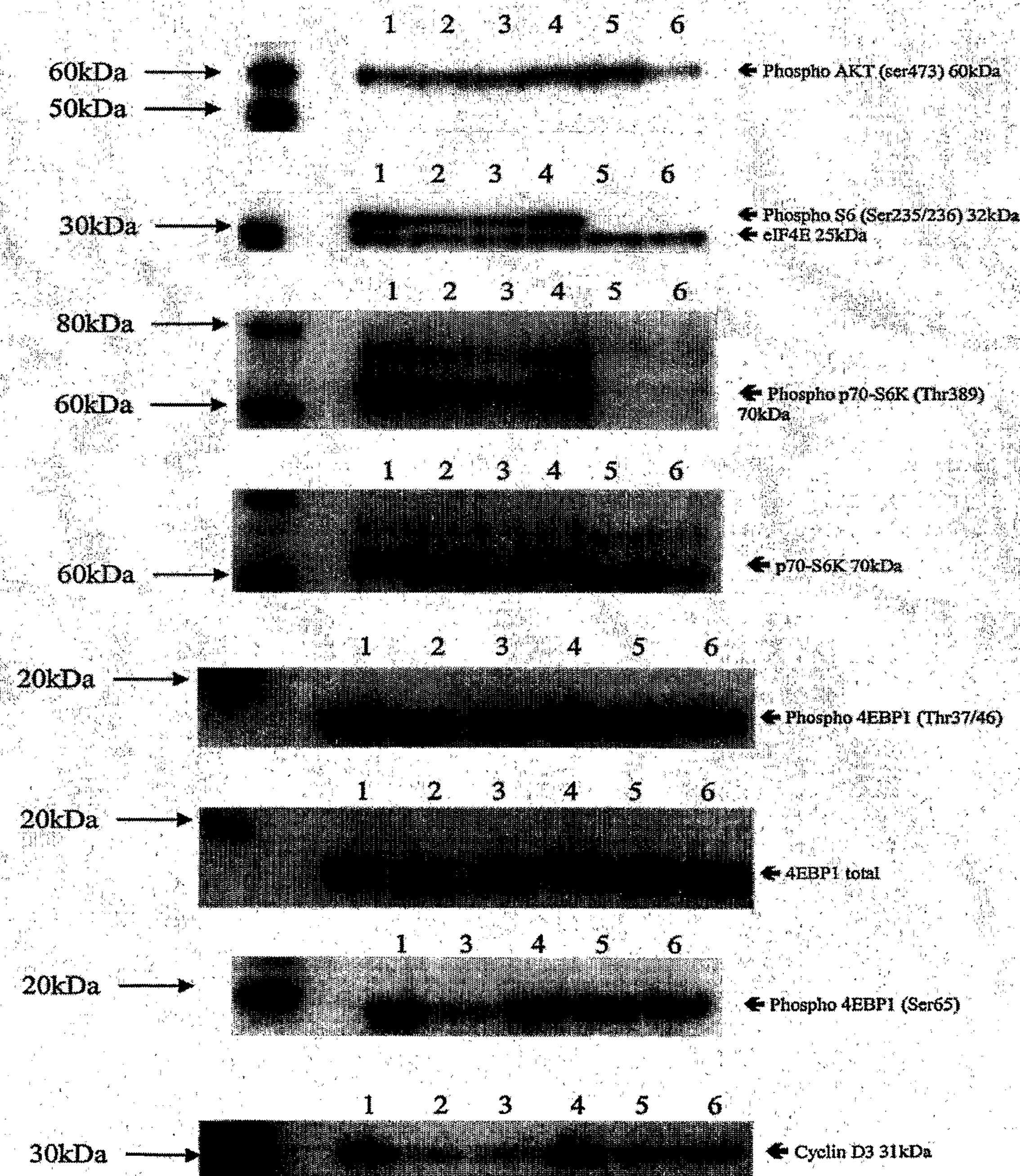


Figure 7: Comparison of MDA468 & MDA 231 cells (48 hours incubation)

1: DMSO	0,08%
2: BIC0043901	200 nM
4: other	2 µM
5: Rapamycin	100 nM
6: LY294002	10 µM

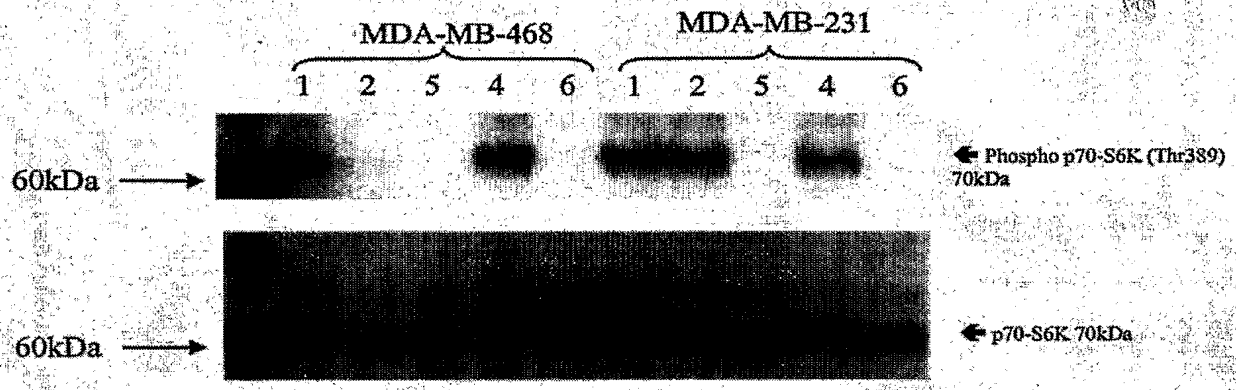


Figure 8